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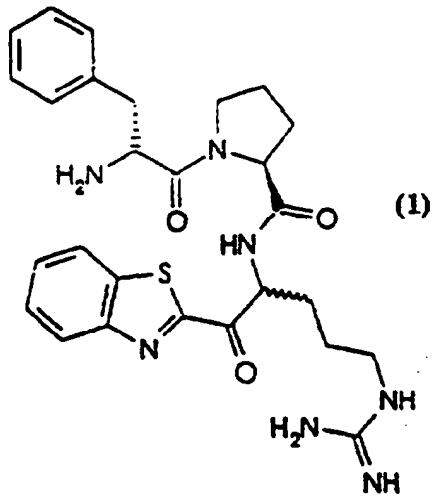
(54) Title: HETEROCYCLIC KETO ARGININE PEPTIDES AS THROMBIN INHIBITORS

(57) Abstract

This invention relates to new and useful inhibitors of the enzyme thrombin of the formula (I): AS - X, and more particularly compound (1) in the preparation, and pharmaceutical compositions. As well, this invention relates to the use of such compounds and compositions in vitro as anticoagulants and in vivo as agents for the treatment and prophylaxis of thrombotic disorders such as venous thrombosis, pulmonary embolism and arterial thrombosis resulting in acute ischemic events such as myocardial infarction or cerebral infarction. Moreover, these compounds and compositions have therapeutic utility for the prevention and treatment of coagulopathis associated with coronary bypass operations as well as restenotic events following transluminal angioplasty.



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HETEROCYCLIC KETO ARGININE PEPTIDES AS THROMBIN INHIBITORS

FIELD OF THE INVENTION

This invention relates to compounds useful for the treatment of thrombotic disorders, and more particularly to novel heterocyclic inhibitors of the enzyme thrombin.

BACKGROUND

Inordinate thrombus formation on blood vessel walls precipitates acute cardiovascular disease states that are the leading cause of death in economically developed societies. Plasma proteins such as fibrinogen, proteases and cellular receptors participating in hemostasis have emerged as important factors that play a role in acute and chronic coronary disease as well as cerebral artery disease by contributing to the formation of thrombus or blood clots that effectively diminish normal blood flow and supply. Vascular aberrations stemming from primary pathologic states such as hypertension, rupture of atherosclerotic plaques or denuded endothelium, activate biochemical cascades that serve to respond and repair the injury site. Thrombin is a key regulatory enzyme in the coagulation cascade. It serves a pluralistic role as both a positive and negative feedback regulator. However, in pathologic conditions the former is amplified through catalytic activation of cofactors required for thrombin generation as well as activation of factor XIII necessary for fibrin cross-linking and stabilization.

In addition to its direct effect on hemostasis, thrombin exerts direct effects on diverse cell types that support and amplify pathogenesis of arterial thrombus disease. The enzyme is the strongest activator of platelets causing them to aggregate and release substances (e.g. ADP TXA_2 NE) that further propagate the thrombotic cycle. Platelets in a fibrin mesh comprise the principal framework of a white

thrombus. Thrombin also exerts direct effects on endothelial cells causing release of vasoconstrictor substances and translocation of adhesion molecules that become sites for attachment of immune cells. In addition, the enzyme causes mitogenesis of smooth muscle cells and proliferation of fibroblasts. From this analysis, it is apparent that inhibition of thrombin activity constitutes a viable therapeutic approach towards the attenuation of proliferative events associated with thrombosis.

The principal endogenous neutralizing factor for thrombin activity in mammals is antithrombin III (ATIII), a circulating plasma macroglobulin having low affinity for the enzyme. Heparin exerts clinical efficacy in venous thrombosis by enhancing ATIII/thrombin binding through catalysis. However, heparin also catalyzes inhibition of other proteases in the coagulation cascade and its efficacy in platelet-dependent thrombosis is largely reduced or abrogated due to inaccessibility of thrombus-bound enzyme. Adverse side effects such as thrombocytopenia, osteoporosis and triglyceridemia have been observed following prolonged treatment with Heparin.

Hirudin, derived from the glandular secretions of the leech *Hirudo medicinalis* is one of the high molecular weight natural anticoagulant protein inhibitors of thrombin activity (Markwardt F. Cardiovascular Drug Reviews, 10, 211, 1992). It is a biopharmaceutical that has demonstrated efficacy in experimental and clinical thrombosis. A potential drawback to the use of hirudin as a therapeutic agent is its weak antigenicity and lack of an effective method of neutralization, especially in view of its extremely tight binding characteristics toward thrombin. The exceedingly high affinity for thrombin is unique and is attributed to a simultaneous interaction with the catalytic site as well as a distal "anion binding exosite" on the enzyme.

Thrombin activity can also be abrogated by hirudin-like molecules such as hirulog (Maraganore, J.M. et al., Biochemistry, 29, 7095, 1990) or hirutonin peptides (DiMaio, J. et al., J. Med. Chem., 35, 3331, 1992).

Thrombin activity can also be inhibited by low molecular weight compounds that compete with fibrinogen for thrombin's catalytic site, thereby inhibiting proteolysis of that protein or other protein substrates such as the thrombin receptor. A common strategy for designing enzyme inhibitory compounds relies on mimicking the specificity inherent in the primary and secondary structure of the enzyme's natural substrate. Thus, Blomback et al. first designed a thrombin inhibitor that was modeled upon the partial sequence of the fibrinogen Aα chain comprising its proteolytically susceptible region (Blomback, et al., J. Clin. Lab. Invest., 24, 59, 1969). This region of fibrinogen minimally includes the residues commencing with phenylalanine:

Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly
-Gly-Gly-Val-Arg-Gly-Pro-Arg
↑ scissile bond

Systematic replacement of amino acids within this region has led to optimization of the tripeptidyl inhibitory sequence exemplified by the peptide (D)-Phe-Pro-Arg which corresponds to interactions within the P_3-P_2-P_1 local binding sites on thrombin (Bajusz S. et al. in Peptides: Chemistry Structure and Biology: Proceedings of the Fourth American Peptide Symposium, Walter R., Meienhofer J. Eds. Ann Arbor Science Publishers Inc., Ann Arbor MI, 1975, pp 603).

Bajusz et al. have also reported related compounds such as

(D)Phe-Pro-Arg-(CO)H (GYKI-14166) and (D)MePhe-Pro-Arg-(CO)H (GYKI-14766) (Peptides-Synthesis, Structure and Function: Proceedings of the Seventh American Peptide Symposium, Rich, D.H. & Gross, E. eds., Pierce Chemical Company , 1981, pp. 417). These tripeptidyl aldehydes are effective thrombin inhibitors both *in vitro* and *in vivo*. In the case of both GYKI-14166 and GYKI-14766, the aldehyde group is presumed to contribute strongly to inhibitory activity in view of its chemical reactivity toward thrombin's catalytic Ser_{195} residue, generating a hemiacetal intermediate.

Related work in the area of thrombin inhibitory activity has exploited the basic recognition binding motif engendered by the tripeptide (D)Phe-Pro-Arg while incorporating various functional or reactive groups in the locus corresponding to the putative scissile bond (i.e. P_1-P_1').

In U.S. Patent 4,318,904, Shaw reports chloromethyl-ketones (PPACK) that are reactive towards Ser_{195} and His_{57}. These two residues comprise part of thrombin's catalytic triad (Bode, W. et al., EMBO Journal 8, 3467, 1989).

Other examples of thrombin inhibitors bearing the (D)Phe-Pro-Arg general motif are those incorporating COOH-terminal boroarginine variants such as boronic acids or boronates (Kettner, C. et al., J. Biol. Chem., 268, 4734, 1993).

Still other congeners of this motif are those bearing phosphonates (Wang, C-L J., Tetrahedron Letters, 33, 7667, 1992) and α-Keto esters (Iwanowicz, E.J. et al.,Bioorganic and Medicinal Chemistry Letters, 12, 1607, 1992).

Neises, B. et al. have described a trichloromethyl ketone thrombin inhibitor (MDL-73756) and Attenburger, J.M. et al. have revealed a related difluoro alkyl amide ketone (Tetrahedron Letters, 32, 7255, 1991).

Maraganore et al. (European 0,333,356; WO 91/02750; U.S. 5,196,404) disclose a series of thrombin inhibitors that incorporate the D-Phe-Pro- moiety and hypothesize that this preferred structure fits well within the groove adjacent to the active site of thrombin. Variations on these inhibitors are essentially linear or cyclic peptides built upon the D-Phe-Pro moiety.

Another series of patents and patent applications have described attempts to develop effective inhibitors against thrombosis by using alpha-ketoamides and peptide aldehyde analogs (EP 0333356;WO 93/15756; WO 93/22344; WO 94/08941; WO 94/17817, EP 0479489, U.S. 5,380,713).

Still others have focused their attention on peptides, peptide derivatives, peptidic alcohols, or cyclic peptides as anti-thrombotic agents (WO 93/22344, EP 0276014; EP 0341607; EP 0291982). Others have examined amindine sulfonic acid moieties to achieve this same end (U.S. 4,781,866), while yet others have examined para or meta substituted phenlyalanine derivatives (WO 92/08709; WO 92/6549).

Many of the examples cited above are convergent by maintaining at least a linear acyclic tripeptidyl motif consisting of an arginyl unit whose basic side chain interacts with a carboxylate group located at the base of the P_1 specificity cleft in thrombin. Two adjacent hydrophobic groups provide additional binding through favorable Van der Waals interactions within a contiguous hydrophobic cleft on the enzyme surface designated the P_3-P_2 site.

An object of the present invention is to provide compounds that display inhibitory activity towards thrombin.

SUMMARY OF THE INVENTION

An aspect of the present invention relates to peptide derivatives represented by formula (I), and pharmaceutically acceptable salts thereof

AS - X

(I)

wherein
X is one or more aromatic or non-aromatic heterocycle unsubstituted or substituted with one or more amino, oxygen, alkyl, aralkyl, or aryl; and
AS is an active site inhibitor of thrombin having an argininyl residue or an analogue thereof connected to **X**.

In another aspect of the present invention, there is provided the use of a compound of formula (I) in the manufacture of a medicament for the treatment of vascular diseases in a mammal including human.

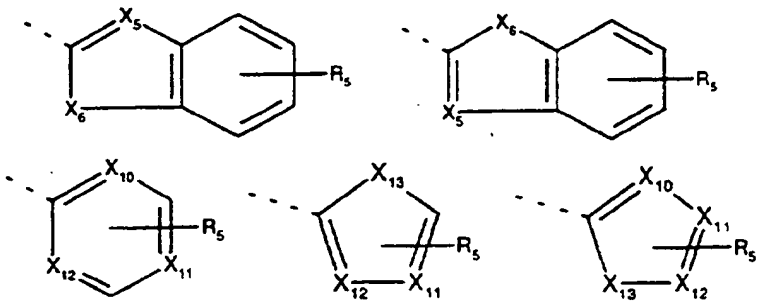
In a further aspect, there is provided a method for the treatment of vascular diseases in a mammal including humans, comprising administering to said mammal an amount of a compound of formula (I) effective to treat vascular diseases.

DETAILED DESCRIPTION OF THE INVENTION

Compounds of the present invention include those compounds where **X** is one or more heterocycle which may be unsubstituted or substituted with amino, oxygen, alkyl,

aralkyl, or aryl. **X** includes aromatic or non-aromatic heterocyclic rings. **X** also includes one or more heterocycle which is optionally fused to another carbocycle or heterocycle.

Preferably **X** is selected from the group consisting of:



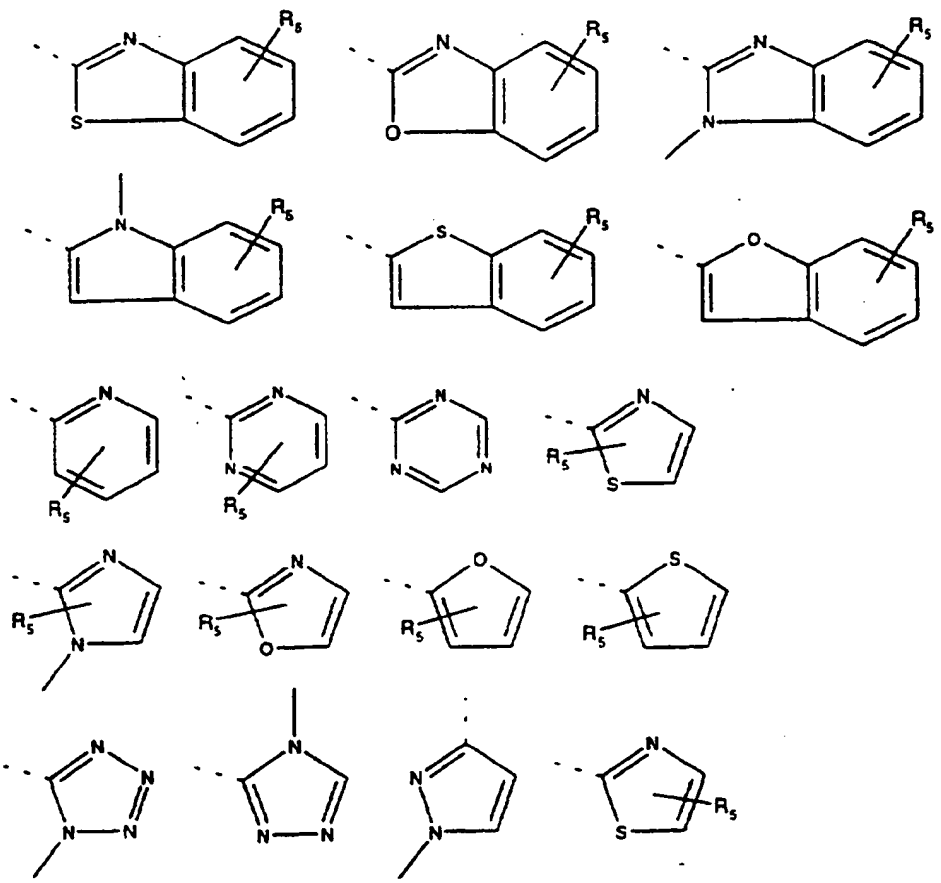
wherein

X_5, X_{10}, X_{11}, and X_{12} are each independently selected from the group consisting of N, or C-X_7 where X_7 is hydrogen, C_{1-4} alkyl, or C_{5-8} aryl.

X_6, and X_{13} are each independently selected from the group consisting of C, O, N, S, N-X_7, or CH-X_7 where X_7 is as defined above.

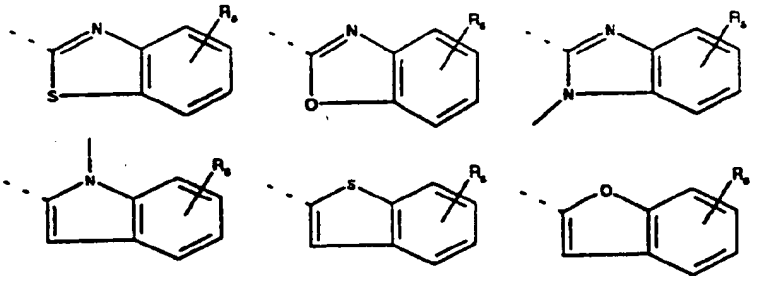
R_5 is hydrogen, C_{1-16} alkyl optionally carboxyl substituted, carboxyl, $-C_{0-16}$ alkyl-CO_2-C_{1-16} alkyl, C_{6-20} aralkyl, C_{3-7} cycloalkyl, aryl or an aromatic heterocycle.

More preferably **X** is selected from the group consisting of:



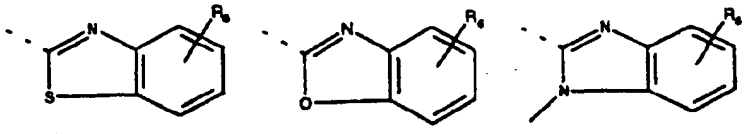
wherein R_5 is as defined above.

Further preferably X is selected from the group consisting of:



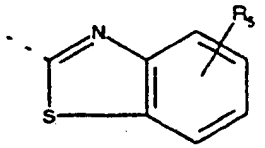
wherein R_5 is as defined above.

Even further preferably X is selected from the group consisting of:

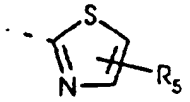


wherein R_5 is as defined above.

Most preferably X is



or



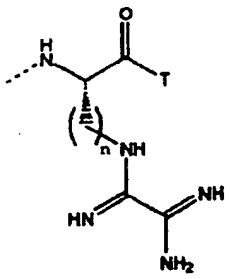
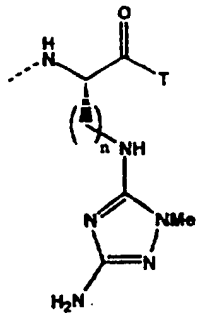
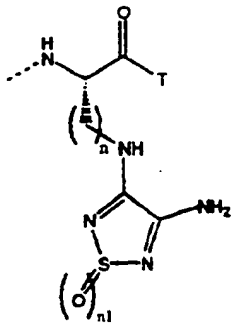
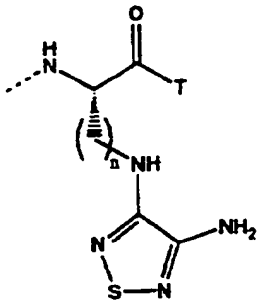
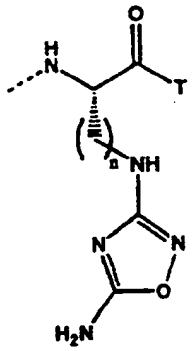
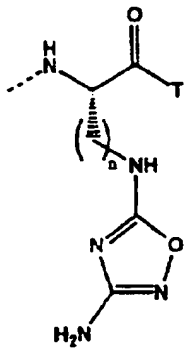
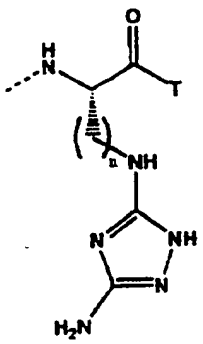
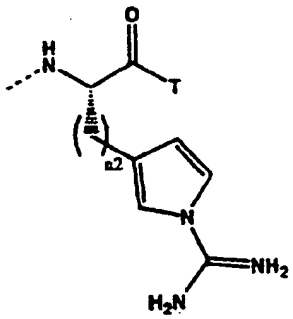
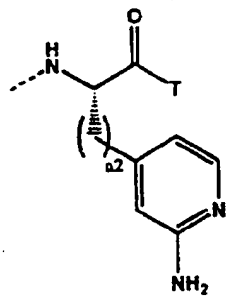
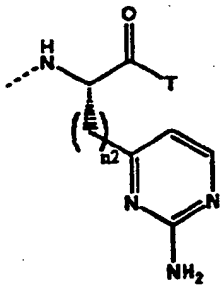
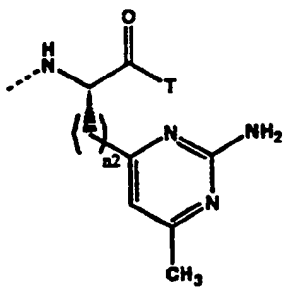
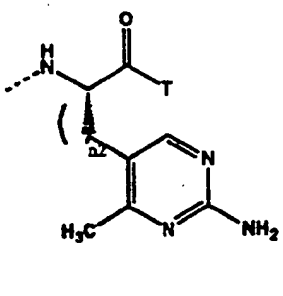
wherein R_5 is as defined above. In another embodiment, X is a 1,2 thiazole optionally substituted with R_5 and\or is attached to J at the 2, 3, 4 or 5 position of the ring.

Preferably, R_5 is hydrogen, or C_{1-4} alkyl.
Further preferably, R_5 is hydrogen or CH_3.
Most preferably, R_5 is hydrogen.

Preferred compounds of formula (I) include those wherein the
AS portion has the formula (II):

$$G^1 - G^2 - \qquad \text{(II)}$$

wherein G^1 is one or more amino acid, alkyl, aryl, aralkyl, or cycloalkyl.
G^2 is arginyl radical or an analogue thereof;
with the proviso that AS is an inhibitor of the active site of thrombin. In particular embodiments G^2 is selected from the following amino acid derivatives prepared according to the procedures described in Bioorg. Med. Chem., 1995, 3:1145.

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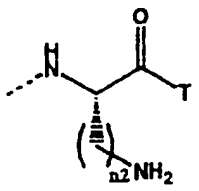
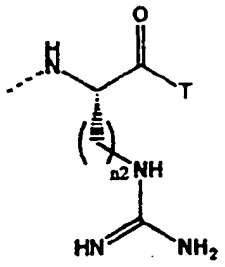
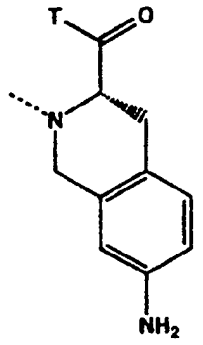
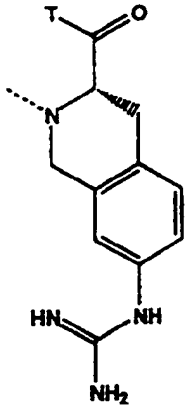
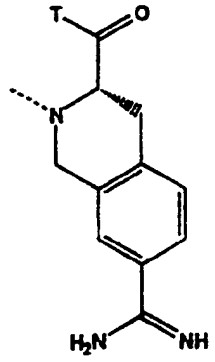
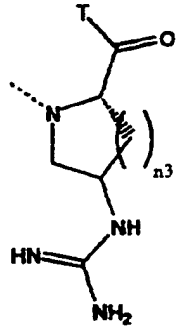
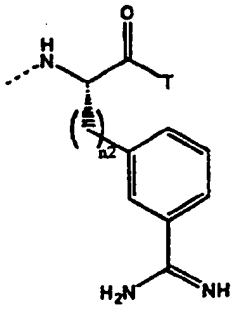
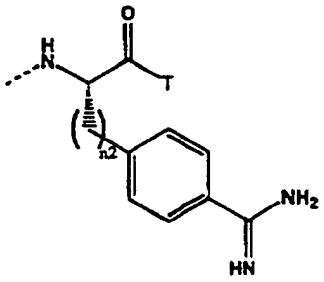
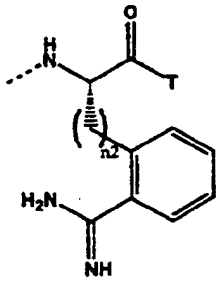

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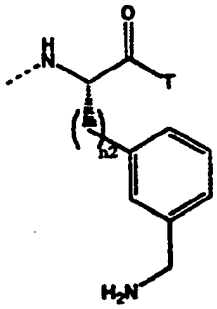
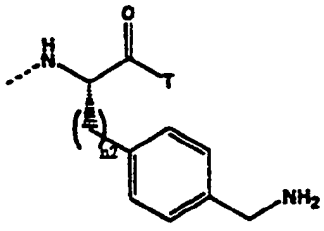
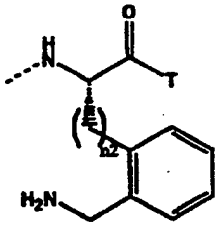
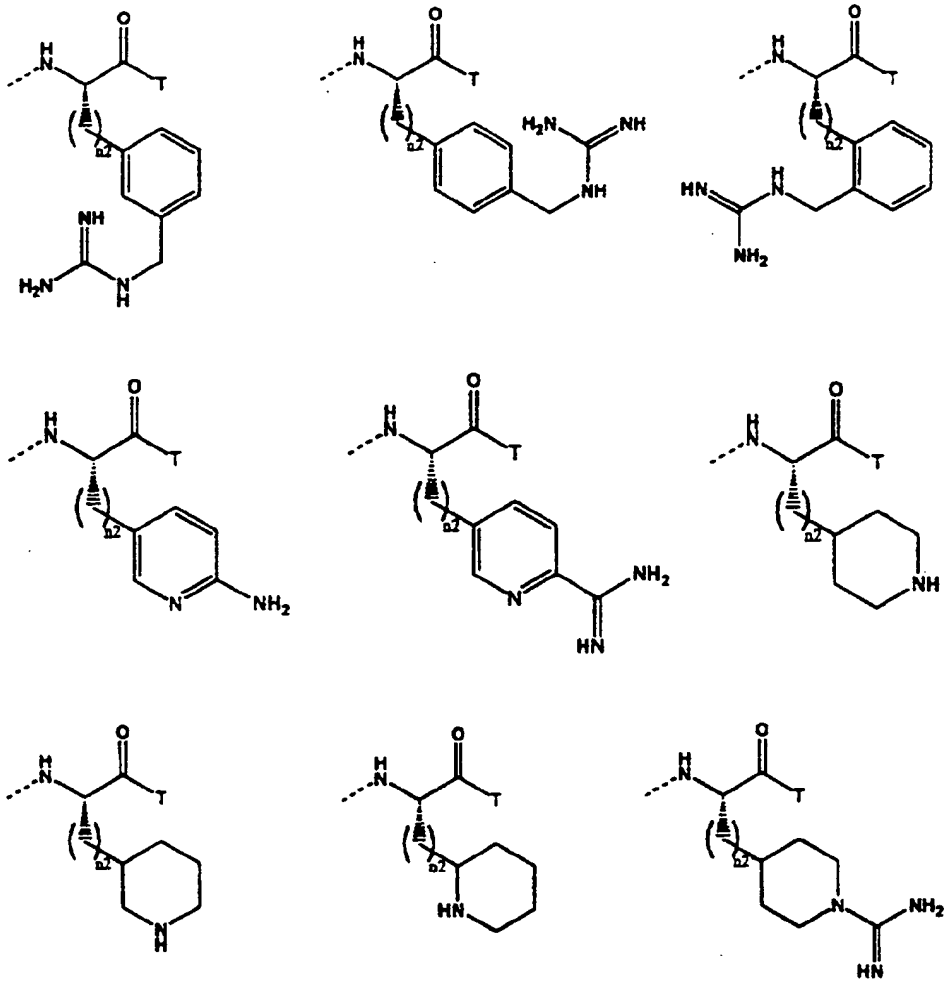
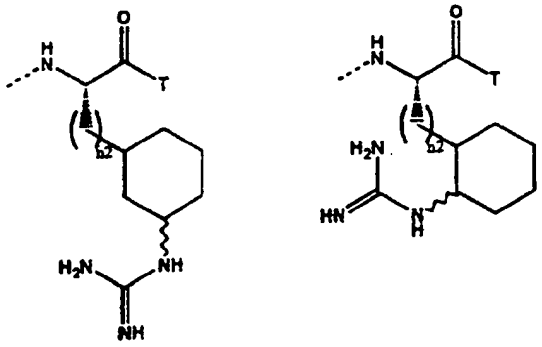








wherein n=1-6, n1=1-2, n2=0-7 and T is a bond or a divalent linking moiety with X.

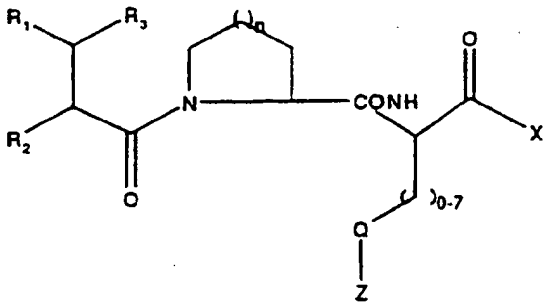
Suitable **AS** portions include amino acids 45-47 of hirudin and analogues thereof, and inhibitors of thrombin based on the D-Phe-Pro-Arg sequence and its analogues such as D-Cha-Pro-Arg, D-Phe-Pip-Arg, and D-Cha-Pip-Arg. Other inhibitors of the active site of thrombin which include an argininyl or an analogue thereof at the C-terminus may also be incorporated into formula (I) as **AS**.

More preferrably, compounds of the present invention include those compounds where **AS** is -Phe-Pro-Arg- or an analogue thereof.

Most preferably compounds of the present invention include those compounds where **AS** is (D-Phe)-Pro-Arg- or an analogue thereof.

It will be appreciated that compounds of the invention encompass all isomers, enantiomers, and mixtures thereof.

In a preferred embodiment, compounds of the invention are represented by formula (III):



(III)

wherein

R_1 is selected from the group consisting of one or more aryl or cycloalkyl which is unsubstituted or substituted with hydroxy, C_{1-6} alkyl, C_{4-8} aralkyl, C_{3-8} aryl, or C_{3-8} cycloalkyl.

R_2 is selected from the group consisting of hydrogen, hydroxy, C_{1-6} alkyl, C_{4-8} aralkyl, and unsubstituted or substituted amino group.

R_3 is selected from the group consisting of hydrogen, hydroxy, SH, C_{1-6} alkyl, C_{3-8} aryl and C_{4-8} aralkyl.

n is an integer from 0 to 2.

Q is a bond or -NH-;

Z is C_{1-4} alkoxy; cyano; $-NH_2$; $-CH_2-NH_2$; $-C(NH)-NH_2$; $-NH-C(NH)-NH_2$; $-CH_2-NH-C(NH)-NH_2$; a C_6 cycloalkyl or aryl substituted with cyano, $-NH_2$, $-CH_2-NH_2$, $-C(NH)-NH_2$, $-NH-C(NH)-NH_2$ or $-CH_2-NH-C(NH)-NH_2$; or a 5 or 6 member, saturated or unsaturated heterocycle optionally substituted with cyano, $-NH_2$, $-CH_2-NH_2$, $-C(NH)-NH_2$, $-NH-C(NH)-NH_2$ or $-CH_2-NH-C(NH)-NH_2$; and

X is as defined above.

Preferred embodiments of the present invention include compounds of formula (III) wherein R_1 is selected from the group consisting of one or more 5 or 6 membered aromatic or non-aromatic ring which may be unsubstituted or substituted with hydroxy, C_{1-4} alkyl, or C_{3-8} cycloalkyl. More preferably R_1 is a 6 membered aromatic or non-aromatic ring unsubstituted or substituted with C_{1-4} alkyl.

Most preferably R_1 is phenyl unsubstituted or substituted with C_{1-4} alkyl.
Most preferably R_1 is phenyl.

Preferably R_2 is hydrogen, hydroxy, C_{1-6} alkyl, or amino unsubstituted or substituted with hydroxy, or C_{1-6} alkyl.
More preferably R_2 is hydroxy or NH_2.
Most preferably R_2 is NH_2.

Preferably R_3 is hydrogen, hydroxy, SH, or C_{1-6} alkyl.
More preferably R_3 is hydrogen, or C_{1-4} alkyl.
Most preferably R_3 is hydrogen.

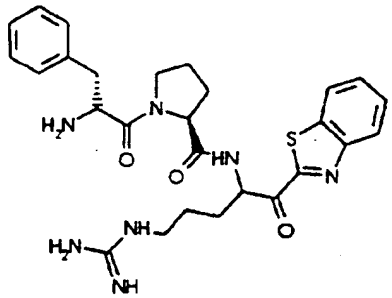
Preferably **n** is 1 or 2.
Most preferably **n** is 1.

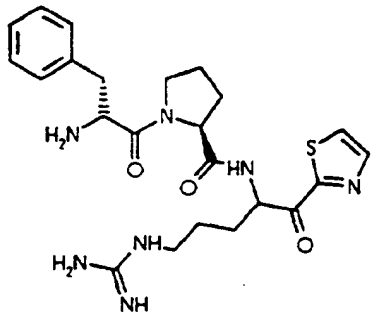
Preferably **Q** is a bond.

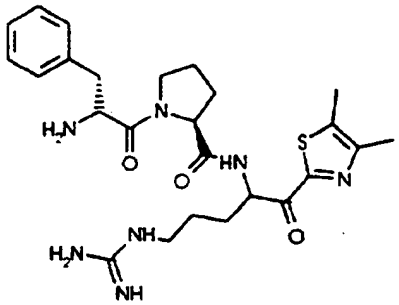
Preferably **Z** is linked via a methylene chain or 2-5 carbon atoms and is selected from the group consisting of $-NH_2$; $-C(NH)-NH_2$; $-NH-C(NH)-NH_2$; a C_6 cycloalkyl or aryl substituted with $-NH_2$, $-CH_2-NH_2$, $-C(NH)-NH_2$, $-NH-C(NH)-NH_2$ or $-CH_2-NH-C(NH)-NH_2$; and a 5 or 6 member, saturated or unsaturated heterocycle optionally substituted with $-NH_2$, $-CH_2-NH_2$, $-C(NH)-NH_2$, $-NH-C(NH)-NH_2$ or $-CH_2-NH-C(NH)-NH_2$.

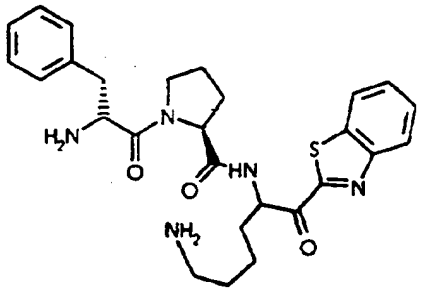
More preferably **Z** is $-NH-C(NH)-NH_2$, $-NH_2$, and $-C(NH)-NH_2$ linked via a methylene chain of 3-5 carbon atoms. Most preferably, Z is $-NH-C(NH)-NH_2$ linked via a trimethylene chain.

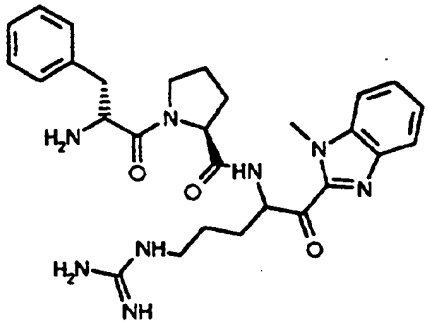
Preferred compounds of the invention include:

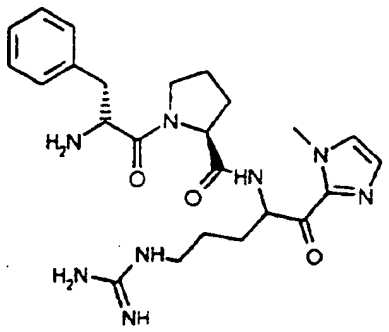












More preferred compounds of formula (I) include:
(D-Phe)-Pro-alpha-benzothiazolo keto arginine; and
(D-Phe)-Pro-alpha-thiazolo keto arginine.

The following abbreviations are referred to herein. These abbreviations are common and well known to those skilled in the art of peptide chemistry.

BOC - butoxy-carbonyl	BuLi - butyl lithium
DCM - dichloromethane	DMF - dimethylformamide
iPr2NEt - diisopropylethylamine	THF - tetrahydrofuran

As used in this application, the term "alkyl" represents a saturated or unsaturated, substituted (for example, by a halogen, hydroxyl, amino, oxygen, sulfur, or C_{6-20} aryl) or unsubstituted, straight chain, branched chain hydrocarbon moiety having 1 to 10 carbon atoms and preferably from 1 to 6 carbon atoms. This chain may be interrupted by one or more heteroatom such as N, O, or S.

The term "amino protecting groups", "oxygen protecting groups", and "protecting groups" are well known in the field of peptide synthesis. Such protecting groups may be found in T. Greene, Protective Groups In Organic Synthesis, (John Wiley & Sons, 1981). The appropriate protecting group for a particular synthetic scheme will depend on many factors, including the presence of other reactive functional groups and the reaction conditions

desired for removal as well known by persons skilled in the art of peptide chemistry.

The term "aryl" represents a carbocyclic moiety which may be substituted by one or more heteroatom (for example N, O, or S) and containing one benzenoid-type ring preferably containing from 6 to 15 carbon atoms (for example phenyl and naphthyl). This carbocyclic moiety may be interrupted by one or more heteroatom such as N, O, or S.

The term "aralkyl" represents an alkyl group being uninterrupted or interrupted , unsubstituted or substituted by an aryl substituent (for example benzyl), preferably containing from 6 to 30 carbon atoms.

Unless specified otherwise, the term "amino acid" used herein includes naturally-occurring amino acids as well as non natural analogs commonly used by those skilled in the art of chemical synthesis and peptide chemistry. A list of non natural amino acids may be found in "The Peptides", vol. 5, 1983, Academic Press, Chapter 6 by D.C. Roberts and F. Vellaccio. It is to be noted that unless indicated otherwise, the amino acids used in the context of the present invention are those in the L-configuration.

The term "cycloalkyl" represents cyclic hydrocarbon groups containing 3 to 12 carbon, preferably 3 to 8 carbon, which includes for example cyclopropyl, cyclobutyl, cyclohexyl, and cyclodecyl, any of which may be substituted with substituents such as halogen, amino, alkyl, and/or hydroxy.

The term "heterocycle" and "heterocyclic rings" represents one or more aromatic or non-aromatic ring which includes one or more heteroatom such as nitrogen, oxygen, and sulfur and which may be substituted with substituents such

as halogen, amino, alkyl, and/or hydroxy. Preferably, the ring is 5, 6, or 7 membered.

While it may be possible that, for use in therapy, a compound of the invention may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising a compound of formula (I) and pharmaceutically acceptable acid addition salt thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

In another aspect of the present invention is provided the use of a compound of formula (I) in the manufacture of a medicament for the treatment of vascular diseases in a mammal including humans.

In another aspect, there is provided a method for the treatment of vascular diseases in a mammal including human, comprising the administration of an effective amount of a compound of formula (I).

It will be appreciated by people skilled in the art that treatment extends to prophylaxis as well to the treatment of established vascular disease.

The compounds of the present invention are useful in combinations, formulations and methods for the treatment and prophylaxis of vascular diseases. These diseases include myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis, peripheral arterial occlusion,

restenosis following arterial injury or invasive cardiological procedures, acute or chronic atherosclerosis, edema and inflammation, cancer and metastasis.

The term "combination" as used herein, includes a single dosage form containing at least one compound of this invention and at least one thrombolytic agent, a multiple dosage form, wherein the thrombin inhibitor and the thrombolytic agent are administered separately, but concurrently, or a multiple dosage form wherein the two components are administered separately, but sequentially. In sequential administration, the thrombin inhibitor may be given to the patient during the time period ranging from about 5 hours prior to about 5 hours after administration of the thrombolytic agent. Preferably, the thrombin inhibitor is administered to the patient during the period ranging from 2 hours prior to 2 hours following administration of the thrombolytic agent.

Thrombolytic agents which may be employed in the combinations of the present invention are those known in the art. Such agents include, but are not limited to, tissue plasminogen activator purified from natural sources, recombinant tissue plasminogen activator, streptokinase, urokinase, purokinase, anisolated streptokinase plasminogen activator complex (ASPAC), animal salivary gland plasminogen activators and known, biologically active derivatives of any of the above.

The dosage and dose rate of the compounds of this invention will depend on a variety of factors, such as the weight of the patient, the specific pharmaceutical composition used, the object of the treatment, i.e., therapy or prophylaxis, the nature of the thrombotic disease to be treated, and the judgment of the treating physician.

According to the present invention, a preferred pharmaceutically effective daily dose of the compounds of this invention is between about 1µg/kg body weight of the patient to be treated ("body weight") and about 5 mg/kg body weight.

Most preferably, the therapeutic and prophylactic compositions of the present invention comprise a dosage of between about 10 µg/kg body weight and about 500 µg/kg body weight of the compounds of this invention. It should also be understood that a daily pharmaceutically effective dose of either the compounds of this invention or the thrombolytic agent present in combinations of the invention, may be less than or greater than the specific ranges cited above.

According to an alternate embodiment of this invention, compounds may be used in compositions and methods for coating the surfaces of invasive devices, resulting in a lower risk of clot formation or platelet activation in patients receiving such devices. Surfaces that may be coated with the compositions of this invention include, for example, prostheses, artificial valves, vascular grafts, stents and catheters. Methods and compositions for coating these devices are known to those of skill in the art. These include chemical cross-linking or physical adsorption of the compounds of this invention-containing compositions to the surfaces of the devices.

According to a further embodiment of the present invention, compounds may be used for ex vivo thrombus imaging in a patient. In this embodiment, the compounds of this invention are labeled with a radioisotope. The choice of radioisotope is based upon a number of well-known factors, for example, toxicity, biological half-life

and detectability. Preferred radioisotopes include, but are not limited to ^{125}I, ^{123}I and ^{111}I. Techniques for labeling the compounds of this invention are well known in the art. Most preferably, the radioisotope is ^{123}I and the labeling is achieved using ^{123}I-Bolton-Hunter Reagent. The labeled thrombin inhibitor is administered to a patient and allowed to bind to the thrombin contained in a clot. The clot is then observed by utilizing well-known detecting means, such as a camera capable of detecting radioactivity coupled to a computer imaging system. This technique also yields images of platelet-bound thrombin and meizothrombin.

This invention also relates to compositions containing the compounds of this invention and methods for using such compositions in the treatment of tumor metastases. The efficacy of the compounds of this invention for the treatment of tumor metastases is manifested by the inhibition inhibitors to inhibit thrombin-induced endothelial cell activation. This inhibition includes the repression of platelet activation factor (PAF) synthesis by endothelial cells. These compositions and methods have important applications in the treatment of diseases characterized by thrombin-induced inflammation and edema, which is thought to be mediated be PAF. Such diseases include, but are not limited to, adult respiratory distress syndrome, septic shock, septicemia and reperfusion damage. Early stages of septic shock include discrete, acute inflammatory and coagulopathic responses.

This invention also relates to the use of the above-described compounds, or compositions comprising them, as anticoagulants for extracorporeal blood. As used herein, the term "extracorporeal blood" includes blood removed in line from a patient, subjected to extracorporeal treatment, and then returned to the patient in such processes as dialysis procedures, blood filtration, or

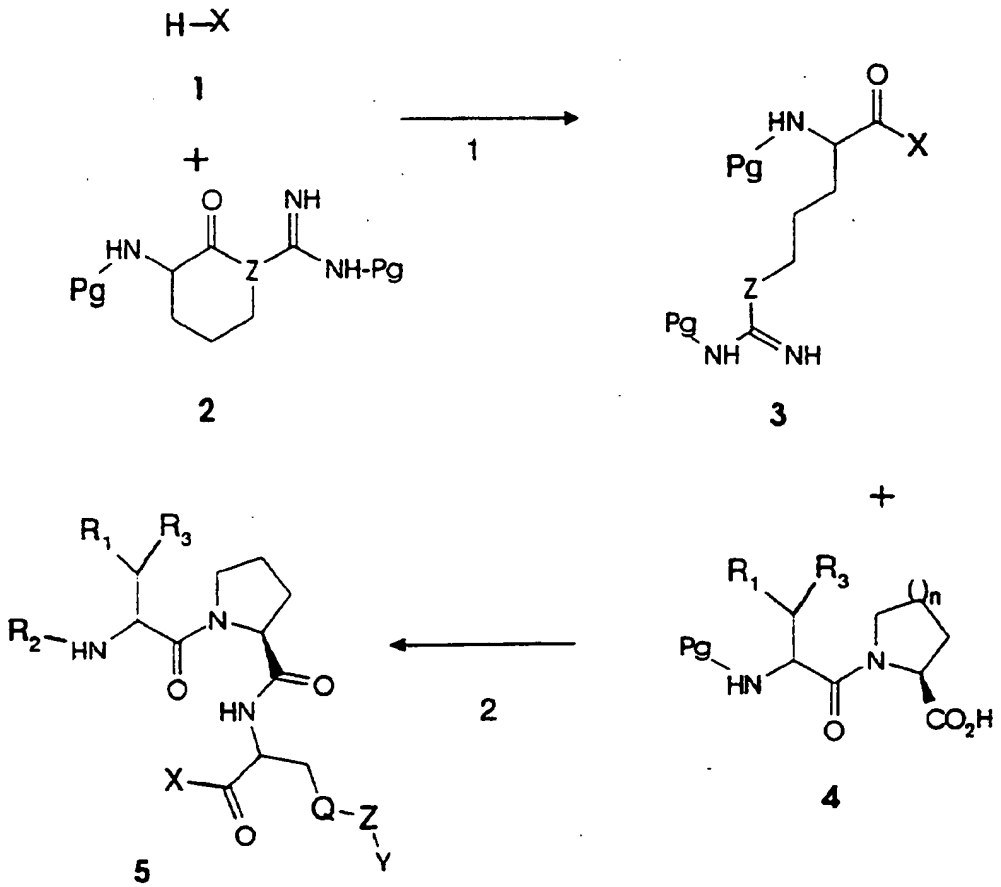
blood bypass during surgery. The term also includes blood products which are stored extracorporeally for eventual administration to a patient and blood collected from a patient to be used for various assays. Such products include whole blood, plasma, or any blood fraction in which inhibition of coagulation is desired.

The amount or concentration of compounds of this invention in these types of compositions is based on the volume of blood to be treated or, more preferably, its thrombin content. Preferably, an effective amount of a compounds of this invention of this invention for preventing coagulation in extracorporeal blood is from about 1 µg/60 ml of extracorporeal blood to about 5 mg/60 ml of extracorporeal blood.

The compounds of this invention may also be used to inhibit clot-bound thrombin, which is believed to contribute to clot accretion. This is particularly important because commonly used anti-thrombin agents, such as heparin and low molecular weight heparin, are ineffective against clot-bound thrombin. Finally, the compounds of this invention may be employed in compositions and methods for treating neurodegenerative diseases. Thrombin is known to cause neurite retraction, a process suggestive of the rounding in shape changes of brain cells and implicated in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.

Compounds of the present invention may be synthesized by various methods well known in the art. Suitable methods of synthesis will vary depending upon the **AS** and **X** portions used in the compound. Suitable methods for synthesis of Phe-Pro-Arg type analogues are described below. However, other well known methods may be employed.

SCHEME 1



Step 1:

The heterocyle 1 in solution was metalated with an appropriate metalating base such as n-BuLi to generate the corresponding metalated heterocylic compound. The cyclic activated arginine group 2 was added to this mixture. Compound 2 was prepared according to procedures known in the literature and described in, for example, R.T. Shuman, *et al.*, "Highly Selective Tripeptide Thrombin Inhibitors", J.Med.Chem, 1993, 36, 314. The compound yielded was heterocyclic ketoarginine 3.

Step 2:

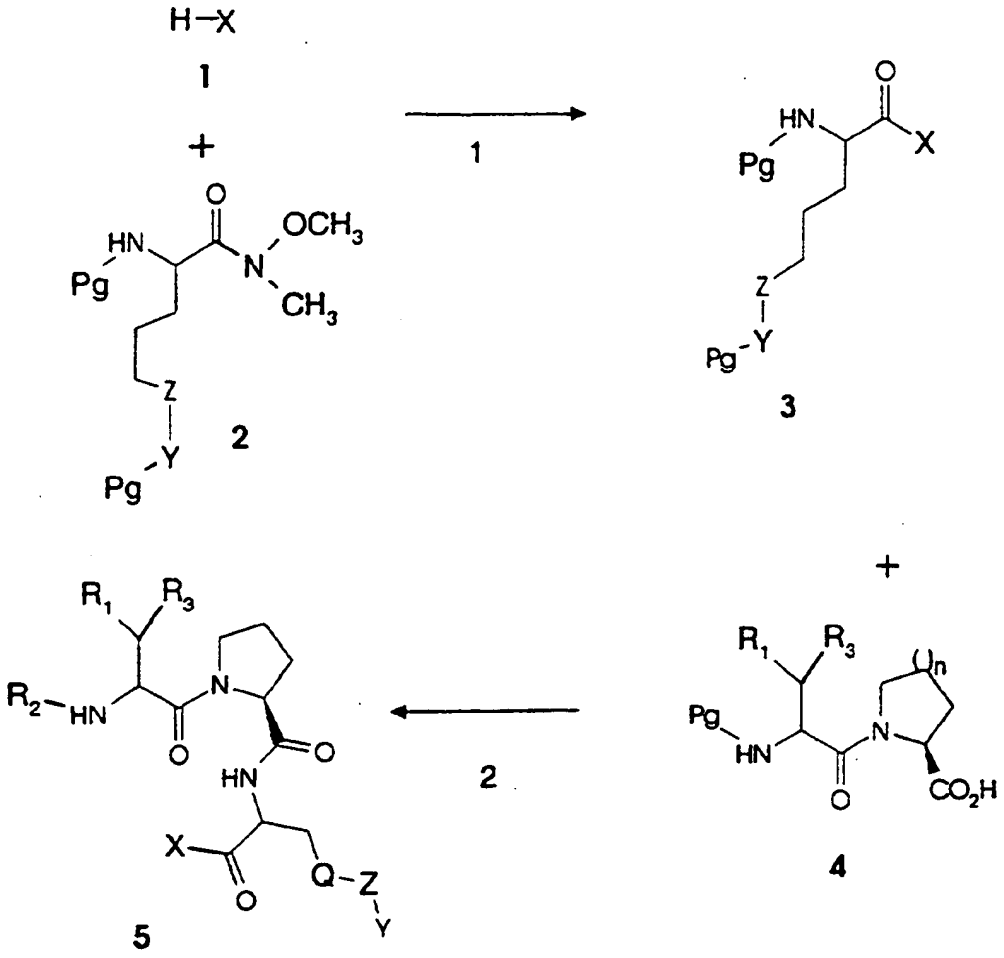
The heterocyclic ketoarginine 3 is deprotected and coupled to the dipeptide 4 in the presence of a suitable coupling

agent, solvent, and base. The dipeptide 4 can be purchased or prepared by methods common in the art and the peptide literature. Suitable coupling agents include BOP and isopropylchloroformate. Suitable solvents include DCM and DMF. Suitable bases include iPr2NEt and n-methyl morpholine.

The resulting compound is deprotected with appropriate deprotecting agents to yield the heterocyclic ketoargininyl 5. Suitable deprotecting agents include BBr_3, HBr in acetic acid, and TMSI. Methods to remove the protecting groups are well known to people skilled in the art.

Scheme I is used where Z is N. Scheme II is used when Z is carbon, linear carbon chain, or forms a ring with Q. Where Z forms a ring with Q, the activated amino group 2 would be amended accordingly to include this ring. The steps in the process remain the same as described for Scheme I.

SCHEME II



The compounds of this invention and their intermediates may be purified during their synthesis and/or after their preparation by standard techniques well known to the skilled artisan. One preferred purification technique is HPLC. However, other chromatographic methods such as column chromatography may be used for purification of the compounds. Crystallization may also be used to purify the products as may washing with appropriate organic solvents.

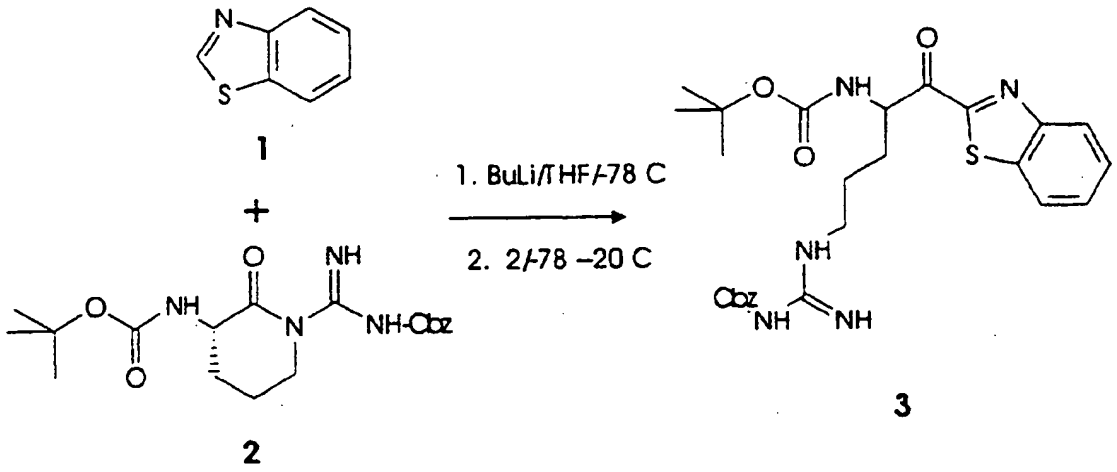
It is well known in the art that the amino protecting groups are not necessary for the reaction to occur. The process may be carried out without protecting groups. However, they are used to increase the yield of the desired compounds.

The process described above may use suitable protecting groups for compounds 2, 3, and 4. Suitable deprotection conditions and protocols are described in the synthesis literature and are well known to chemists skilled in the art.

Desired R_1, R_2, and R_3 groups may be substituted onto the dipeptide 4 before it is coupled to heterocyclic ketoarginine 3 using techniques well known in the art of peptide chemistry. Also, preferred analogues of each of the peptides or the dipeptide may be purchased with the desired R_1, R_2, or R_3 groups substituents already present.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

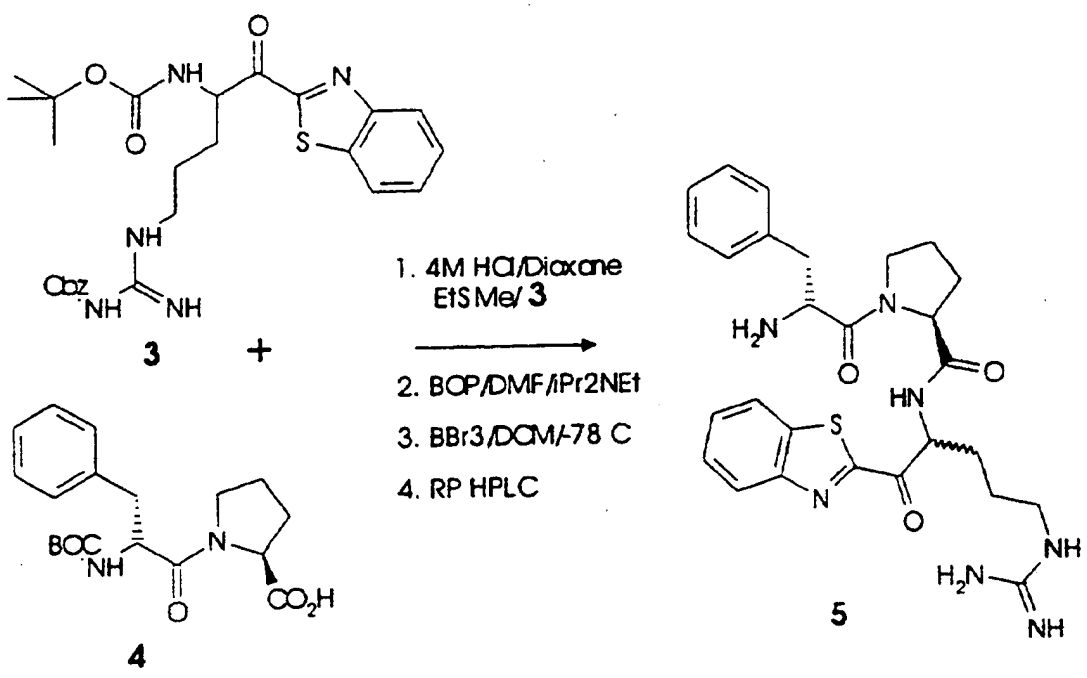
EXAMPLE 1



To a THF (75 mL) solution of benzothiazole (compound 1) (4.0 mL, 36.7 mmol) at -78 °C was slowly added n-BuLi (1.6 M, 25 mL), resultant orange suspension was stirred at -78 °C for 1.5 h. Then added solid compound 2 (3.55 g, 8.7 mmol). Reaction stirred at -78 °C for 30 min followed by at -20 for 30 min then quenched with saturated aqueous NH_4Cl . Extraction with ethyl acetate followed by column chromatography afforded yellow foam (1.28 g) in 28% yield as compound 3.

NMR(CDCl3) d 1.45 (s, 9H), 1.5-1.8 (m, 2H), 3.1-3.23 (m, 1H), 3.45-3.60 (m, 1H), 5.1(d, 2H), 5.53-5.64(m, 2H), 7.02-7.15(m, 4H), 7.21-7.28(m, 2H), 7.56-7.65(m, 2H), 8.0-8.05(m, 1H), 8.18-8.23(m, 1H).

MS: (M+1) 526.8



To a mixture of compound 3 (0.223 g, 0.43 mmol) and EtSMe (0.25 mL), at ambient temperature, was added 4M HCl solution in dioxane (10 mL). The reaction was stirred for 1 h. All the solvents removed and the yellow gummy solid was dried. To this yellow solid was added compound 4 (0.17 g, 0.47 mmol) and BOP (0.21 g, 0.48 mmol) in DMF (5mL) at room temperature then to this mixture was added iPr2NEt until the pH of the mixture reaches 8-9. The reaction was allowed to stir overnight. The reaction was extracted with ethyl acetate and washed with brine, subsequent column chromatography gave 0.129 g of the desired precursor to compound 5 which was dissolved in DCM (10 mL) and added 1M BBr3 solution in DCM (1.7 mL, 1.66 mmol) at -78 °C. Reaction was stirred at -78 °C for 30 min followed by for 3 h at room temperature. Cooled back to -78 °C and added anhd. MeOH (2mL) followed by stirring at RT for 1h. All the solvents removed the mixture extracted with water and washed with ether. The water fraction lyophilized and was subjected to reverse phase HPLC purification to yield compound 5 . The two compounds were isolated as individual

diastereomers analogue 1 and analogue 2 with identical Mass spectra [(M+1) 536.5]

EXAMPLE 2

Determination of K_i Values

This assay was performed with a Perkin Elmer fluorometer model #LS 50B using a fluorogenix thrombin substrate (Tos-Gly-Pro-Arg-AMC.HCl) purchased from Calbiochem. Human thrombin was also obtained from Calbiochem. Measurements were determined at excitation and emission wavelengths of 383 and 455nm respectively.

The assay was carried out in running buffer consisting of 50mM Tris, 100mM NaCl, 0.1% and Peg pH 7.8 at 24°C. Buffer, substrate and inhibitor were mixed and the reaction was initiated by adding the enzyme solution. Initial velocities were recorded at several inhibitor and substrate concentrations. Kinetic parameters were determined by fitting the data to a general equation describing enzyme inhibition (Segel, *Enzyme Kinetics*, Wiley Interscience Publications, 1993).

Dixon and Lineweaver-Burk plots were used to estimate the kinetic parameters (K_m, V_{max}, K_i) using the Microsoft™ Excell™ program.

Binding is the establishment of the equilibria between enzyme, inhibitor, and enzyme-inhibitor complexes. In slow binding inhibition, this equilibrium is established slowly. Equilibrium dissociation constant for compound 5 is shown in Table 1. The result is compared with known reported tripeptidyl based thrombin inhibitors.

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Compound 5 exhibited slow binding kinetics, however the inhibition constant was determined assuming rapid steady state kinetics. Therefore, the reported values are a reliable estimate of the equilibrium inhibitory constants.

dTT assay

Procedure:

Fibrinogen, and buffer solution were transferred to disposable tubes and placed in a water bath for about 15 to 30 minutes before the assay to allow equilibration to 37°C.

The cuvette-strips were incubated for 3 minutes at 37°C. A ball was dispensed to each cuvette. To the prewarmed cuvettes was added 75µl buffer, 50 µl inhibitor solution, and 50 µl fibrinogen solution. The timer was started corresponding to the incubation column for an incubation of 60 seconds. The cuvettes were transferred to the test column area. The multipette was primed once with the start reagent (thrombin solution). The multipette was activated and 25 µl of thrombin solution was dispensed. When the clotting times were determined, they were displayed and printed.

A time versus inhibitor concentrations curve was constructed and IC_{50} values were extrapolated from the inhibitor concentration curves. The IC_{50} is defined as the dose required to double the coagulation time compared to control.

The result showing IC_{50} value is shown in Table 1.

TABLE I

COMPOUND	K_i (nM)	IC_{50}(dTT)(nM)
5	0.05-0.180	1.8-7.2
PPACK	0.017	2.5

Boc-D-Phe-Pro-Arg-H	45
D-1-Tiq-Pro-Arg-H	19

The results in Table I demonstrate that a heterocyclic function such as is embidied in a benzothiazolo-keto-arginyl unit spanning the S_1-S_1' sites of thrombin enhances enzyme affinity up to 1000 fold compared to other reported inhibitors. Compound 5 is equipotent to PPACK which is regarded as an irreversible inhibitor of thrombin that forms a covalent bond with the enzyme whereas compound 5 is a reversible inhibitor of thrombin.

Having now fully described the invention, it will be apparent to one of ordinary skill in the art that numerous modifications can be made thereto without departing from the spirit or the invention as set forth herein.

WE CLAIM:

1. A thrombin inhibiting compound according to formula (I), and pharmaceutically acceptable salts thereof

AS - X

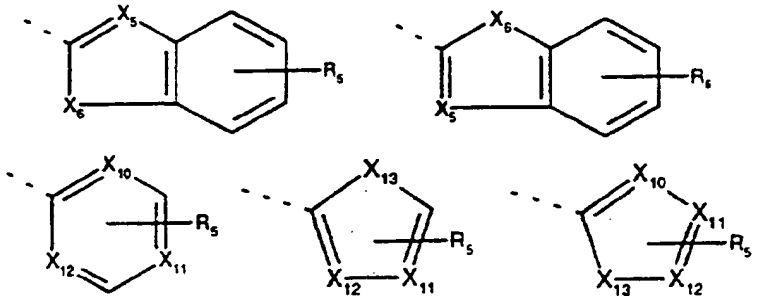
(I)

wherein

X is one or more aromatic or non-aromatic heterocycle unsubstituted or substituted with one or more amino, oxygen, alkyl, aralkyl, or aryl; and

AS is an active site inhibitor of thrombin having an argininyl residue or an analogue thereof connected to **X**.

2. A compound according to claim 1, wherein X is selected from the group consisting of:



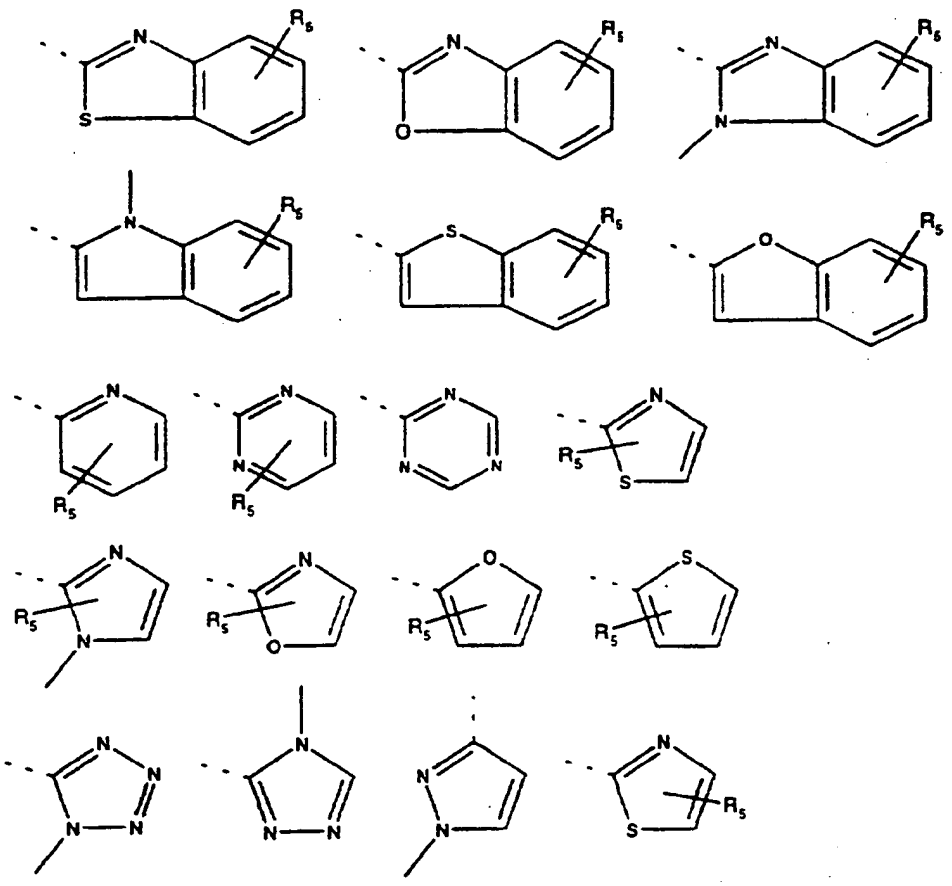
wherein

X_5, X_{10}, X_{11}, and X_{12} are each independently selected from the group consisting of N, or C-X, where X, is hydrogen, C_{1-4} alkyl, or C_{5-8} aryl;

X_6, and X_{13} are each independently selected from the group consisting of C, O, N, S, N-X_7, or CH-X_7; and

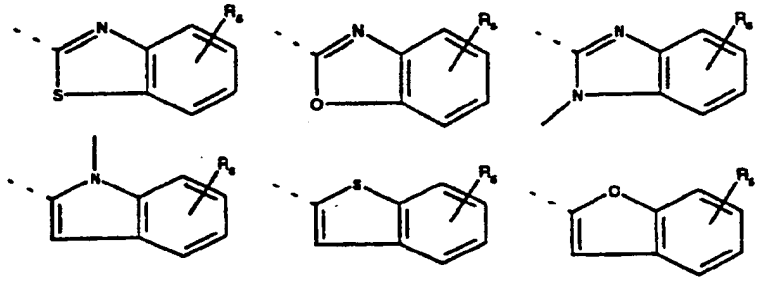
R_5 is hydrogen, C_{1-16} alkyl optionally carboxyl substituted, carboxyl, -C_{0-16} alkyl-CO_2-C_{1-16} alkyl, C_{6-20} aralkyl, C_{3-7} cycloalkyl, aryl or an aromatic heterocycle.

3. A compound according to claim 2, wherein **X** is selected from the group consisting of:



R_5 is hydrogen, C_{1-16} alkyl optionally carboxyl substituted, carboxyl, $-C_{0-16}$ alkyl-CO_2-C_{1-16} alkyl, C_{6-20} aralkyl, C_{3-7} cycloalkyl, aryl or an aromatic heterocycle.

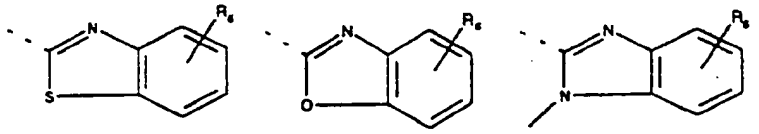
4. A compound accoring to claim 2, wherein X is selected from the group consisting of:



wherein

R_5 is hydrogen, C_{1-16} alkyl optionally carboxyl substituted, carboxyl, $-C_{0-16}$ alkyl-CO_2-C_{1-16} alkyl, C_{6-20} aralkyl, C_{3-7} cycloalkyl, aryl or an aromatic heterocycle.

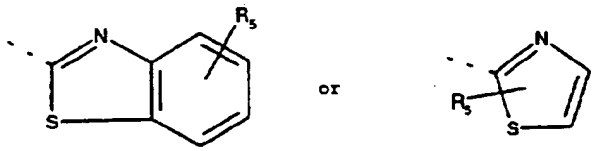
5. A compound according to claim 2 wherein X is selected from the group consisting of:



wherein

R_5 is hydrogen, C_{1-16} alkyl optionally carboxyl substituted, carboxyl, $-C_{0-16}$ alkyl$-CO_2-C_{1-16}$ alkyl, C_{6-20} aralkyl, C_{3-7} cycloalkyl, aryl or an aromatic heterocycle.

6. A compound according to claim 2 wherein X is selected from the group consisting of:



wherein

R_5 is hydrogen, C_{1-16} alkyl optionally carboxyl substituted, carboxyl, $-C_{0-16}$ alkyl$-CO_2-C_{1-16}$ alkyl, C_{6-20} aralkyl, C_{3-7} cycloalkyl, aryl or an aromatic heterocycle.

7. A compound according to claim 1, wherein AS is a group of formula (II):

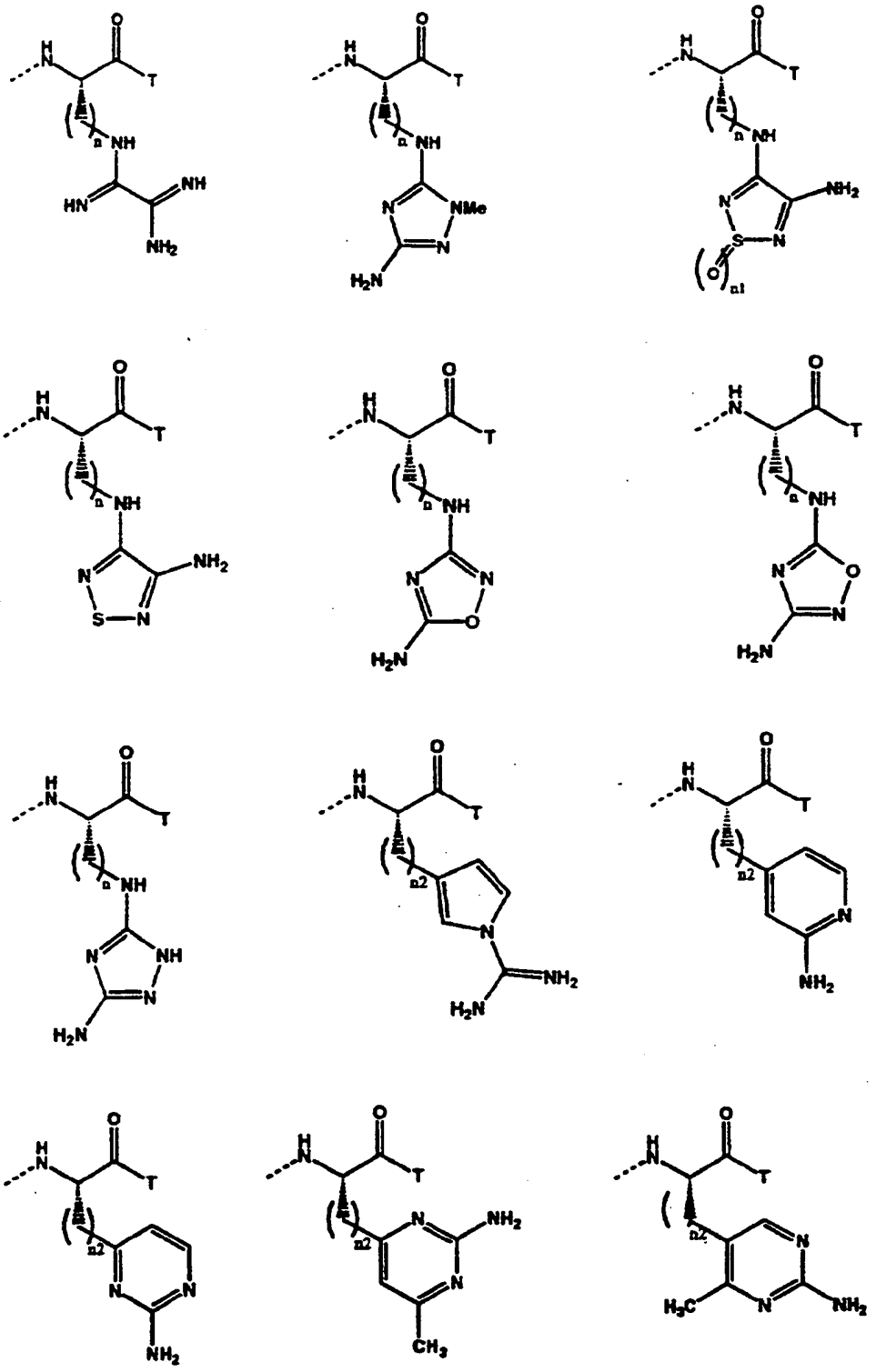
$$G^1 - G^2 -$$

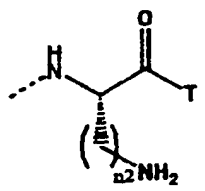
(II)

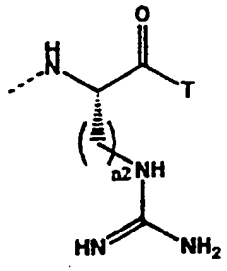
wherein G^1 is one or more amino acid, alkyl, aryl, aralkyl, or cycloalkyl; and

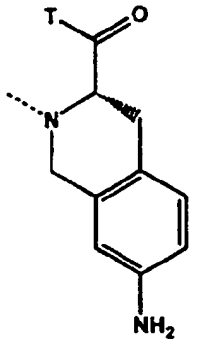
G^2 is arginyl radical or an analogue thereof.

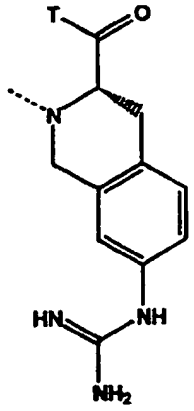
8. A compound accoring to claim 7, wherein G^2 is an arginyl radical selected from:

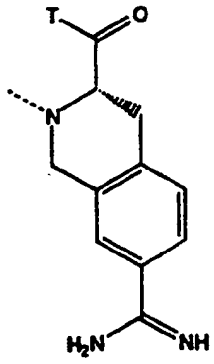


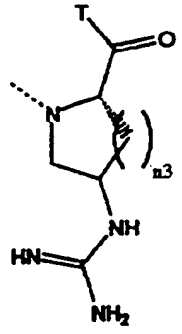


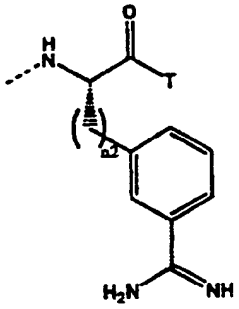


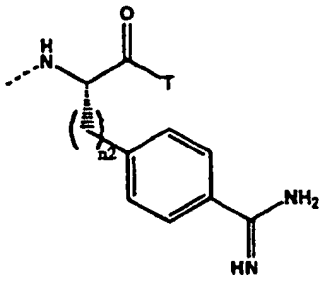


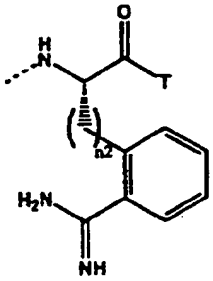












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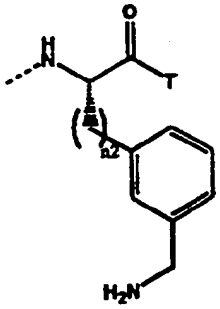

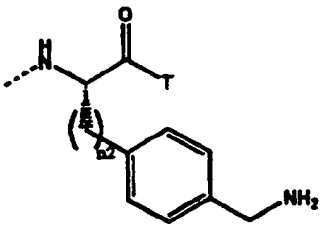

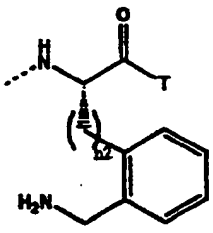

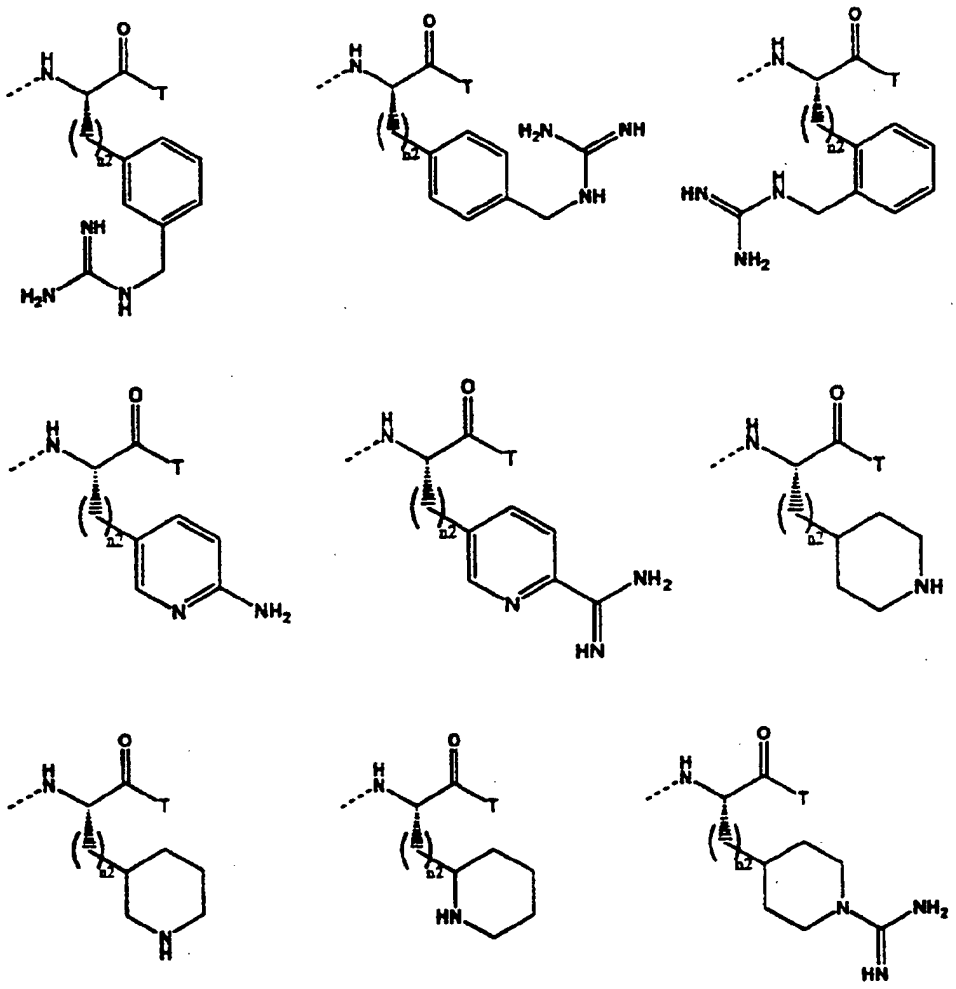

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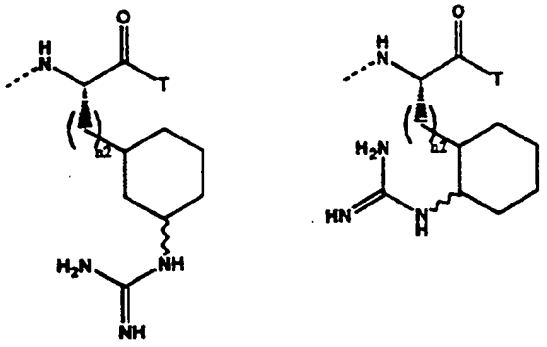
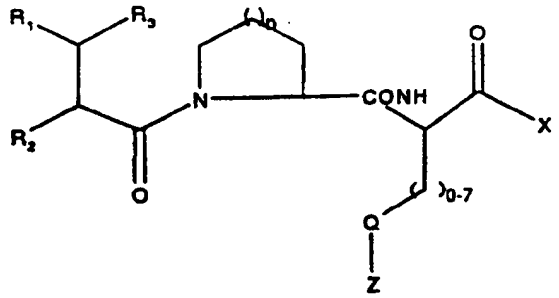


wherein n=1-6, n1=1-2, n2=0-7 and T is a bond or a divalent linking moiety with X.

9. A compound according to claim 1, wherein AS is the peptide fragment of hirudin 45-47 and analogues thereof.

10. A compound according to claim 1, wherein AS is selected from D-Phe-Pro-Arg; D-Cha-Pro-Arg; D-Phe-Pip-Arg; and D-Cha-Pip-Arg.

11. A thrombin inhibiting compound according to formula (III):



(III)

wherein

R_1 is selected from the group consisting of one or more aryl or cycloalkyl which is unsubstituted or substituted with hydroxy, C_{1-6} alkyl, C_{4-8} aralkyl, C_{3-8} aryl, or C_{3-8} cycloalkyl.

R_2 is selected from the group consisting of hydrogen, hydroxy, C_{1-6} alkyl, C_{4-8} aralkyl, and unsubstituted or substituted amino group.

R_3 is selected from the group consisting of hydrogen, hydroxy, SH, C_{1-6} alkyl, C_{3-8} aryl and C_{4-8} aralkyl.

n is an integer from 0 to 2.

Q is a bond or -NH-;

Z is C_{1-4} alkoxy; cyano; $-NH_2$; $-CH_2-NH_2$; $-C(NH)-NH_2$; $-NH-C(NH)-NH_2$; $-CH_2-NH-C(NH)-NH_2$; a C_6 cycloalkyl or aryl substituted with cyano, $-NH_2$, $-CH_2-NH_2$, $-C(NH)-NH_2$, $-NH-C(NH)-NH_2$ or $-CH_2-NH-C(NH)-NH_2$; or a 5 or 6 member, saturated or unsaturated heterocycle optionally substituted with cyano, $-NH_2$, $-CH_2-NH_2$, $-C(NH)-NH_2$, $-NH-C(NH)-NH_2$ or $-CH_2-NH-C(NH)-NH_2$; and

X is one or more aromatic or non-aromatic heterocycle unsubstituted or substituted with one or more amino, oxygen, alkyl, aralkyl, or aryl.

12. A compound according to claim 11, wherein R_1 is selected from the group consisting of one or more 5 or 6 membered aromatic or non-aromatic ring optionally substituted with hydroxy, C_{1-4} alkyl, or C_{3-8} cycloalkyl.

13. A compound according to claim 12, wherein

R_1 is phenyl;

R_2 is hydroxy or NH_2;

R_3 is hydrogen, or C_{1-4} alkyl;

n is 1 or 2;

Q is a bond; and

Z is $-NH-C(NH)-NH_2$, $-NH_2$, and $-C(NH)-NH_2$ linked via a methylene chain of 3-5 carbon atoms.

14. A compound according to claim 1 selected from (D-Phe)-Pro-alpha-benzothiazolo keto arginine and (D-Phe)-Pro-alpha-thiazolo keto arginine.

15. The use of a compound according to any one of claims 1 to 14 in the manufacture of a medicament for the treatment of vascular diseases in a mammal including humans.

16. The use according to claim 15, wherein said vascular disease is thrombosis.

17. A method for the treatment or prophylaxis of thrombotic disorders in a mammal, comprising administering to said mammal an effective amount of a compound according to any of one claims 1 to 14.

18. The method according to claim 17, wherein said disorder is venous thrombosis.

19. The method according to claim 17, wherein said disorder is pumonary embolism.

20. The method according to claim 17, wherein said disorder is arterial thrombosis.

21. The method according to claim 17, wherein said disorder is myocardial infarction.

22. The method according to claim 17, wherein said disorder is cerebral infarction.

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/CA 95/00711

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 C07K5/08 A61K31/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 191 753 (J. W. RYAN) 4 March 1980 see claim 1 ---	14
A	EP,A,0 462 884 (ADIR ET COMPANIE) 27 December 1991 see claim 1 -----	14

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 1996	-3. 04 96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Voyiazoglou, D

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. nal Application No
PCT/CA 95/00711

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4191753	04-03-80	NONE		
EP-A-462884	27-12-91	FR-A-	2663336	20-12-91
		AU-B-	631068	12-11-92
		AU-B-	7844791	19-12-91
		CA-A-	2044736	19-12-91
		DE-T-	69100128	13-01-94
		ES-T-	2059079	01-11-94
		JP-A-	4253995	09-09-92
		OA-A-	9368	15-09-92
		US-A-	5190923	02-03-93